THE STUDY OF SERUM ZINC AND COPPER ALTERATIONS IN INFECTIOUS DISEASES

THESIS

FOR

DOCTOR OF MEDICINE

(PAEDIATRICS)

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1993 **BRIJ MOHAN**

C E R T I F I C A T E

This is to certify that the work entitled "THE STUDY OF SERUM ZINC AND COPPER ALTERATIONS IN INFECTIOUS DISEASES" which is being submitted as THESIS for M.D.(Paediatrics) examination, 1993 of Bundelkhand University by BRIJ MOHAN has been carried out in the Department of Paediatrics, M.L.B. Medical College and Hospital, Jhansi, U.P.

He has put in the necessary stay in the department according to university regulations.

Dated : 16.11.92

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This is to certify that the work entitled "THE STUDY OF SERUM ZINC AND COPPER ALTERATIONS IN INFECTIOUS DISEASES" which is being submitted as THESIS for M.D.(Paediatrics) by BRIJ MOHAN has been carried out under my supervision and guidance. The techniques embodied in the thesis were undertaken by the candidate himself and the observations recorded have been regularly checked by me.

He has fulfilled necessary requirements of the stay in the department for the submition.

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A C K N O W L E D G E M E N T

A C K N O W L E D G E M E N T S

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Brij Mohan

Dated : 16.11.92

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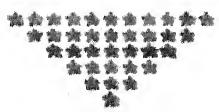
C O N T E N T S



I N T R O D U C T I O N

INTRODUCTION

Importance of inorganic elements in biochemical and physiological processes is now well established at all levels of cellular complexity. Although more than sixty elements have been discovered in bacteria, fungi, higher plants, animals and man, few of them have been studied intensively. In humans, the major elements are carbon, calcium, chloride, hydrogen, iron, nitrogen, magnesium, oxygen, phosphorus, potassium, sodium and sulphur, while minor elements (trace elements or micronutrients) includes aluminium, cadmium, chromium, cobalt, copper, gallium, iodine, molybdenum, nickel, rubidium, selenium, silver, strantium, tin, titanium, vanadium, and zinc.

Both groups of elements are required for anatomical and physiological human growth and development.

Trace elements comprise metals in biological fluids at concentration below one microgram per gram of wet weight. Inspite of their scaricity, most of these are essential nutrients for human beings and are required by the body in minute amounts.

Copper and zinc have many biochemical roles and functions in the body as metalloenzymes, co-enzymes and as a component atoms of physiologically important proteins and hormones.

In the past few years there have been a variable explosion in the basic knowledge about trace element abnormalities in experimental animals as well as in human beings. This information explosion has now reached the stage where clinicians are going to be called upon more frequently to diagnose and treat trace element abnormalities.

The symptoms of deficiency of copper and zinc are now well documented. Three genetically determined disorders have been identified viz., Menkes kinky hair disease (Tricho-poliodystrophy), Wilson's disease (Hepatolenticular degeneration) and acrodermatitis entropathica. Besides these, there are many other clinical disorders in which their role is suspected and is being investigated.

First description of Iranian dwarfs presenting with growth retardation, hypogonadism, hepatosplenomegaly, geophagia, rough skin and anemia (Prasad et al, 1963) as a zinc deficient condition attracted the attention of clinicians for the study of zinc in various disease states.

For more than a century, infection induced alterations have been recognized in the metabolism or body content of many substances. With respect to the trace elements, recent reviews have emphasized the consistent occurente during various infections of changes in ~~same~~ serum concentration and metabolic homeostasis of iron, zinc and copper.

Occasional changes may also occur in the metabolism of maganese, cobalt, gallium, iodine and chromium Vikbladh (1950) was the first to report that serum zinc concentrations were reduced below normal in patients with acute infectious illnesses. His observations have been amply confirmed in patients with bacterial, viral, ricketsial, spirochaetal, and parasitic infections (Beisel et al 1977;Halstead and Smith 1970; Mcbean et al 1972).

Unlike the declines in serum zinc and iron concentrations, serum copper values tend to increase in conjunction with proportionately higher values of the copper binding protein ceruloplasmin (Markowitz et al, 1955).

The progress of an infectious conditions is partly affected by the overal nutrition of the host, the duration of infection, the competence of liver cell functions and the type of therapy. Despite these variables the major trace elements responses are remarkably consistent and can be ascribed to well defined pathophysiological control mechanism. Many of the essential trace elements like copper, zinc, iron and selenium influence the function of the immune system (Chandra and Puri, 1985). Deficiencies of these trace elements can have a detrimental influence on the immune response. Deficiency of zinc produces thymic involution (Golden et al, 1977), decreased activity of T helper cells

and natural killer cell activity, reduced proliferation of lymphocytes in response to mitogens, and delayed cutaneous hypersensitivity (Chandra and Puri, 1985; Good et al, 1982; Golden et al, 1978). Even marginal copper deficiency can lead to an impaired humoral mediated response (Probhaska and Lukasewycz, 1981; Sullivan and Ochs, 1981).

A product of phago cytizing cell leukocyte endogeneus mediaters (LEM) acts on the liver to stimulate an accelerated flux of zinc into hepatic cells and to cause an accelerated synthesis of ceruloplasmin. Other mechanisms that may influence trace elements metabolism include altered body balances, sequestration of the elements within tissues, changes in metal binding transport proteins, harmonal action and trace elements uptake by invading organisms.

Infectious diseases still heads the least responsible for morbidity and mortality in children, Hence the present study was undertaken to observe the influence of some common infectious illnesses, e.g. tuberculosis Enteric fever, Pneumonia, Pyogenic meningitis, hepatitis, Malaria etc on the serum concentration of copper and zinc.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The name of zinc is derived from a German word meaning of unknown origin (1550 BC). Chinese and Indians were having knowledge of extraction of zinc metal as early as Thirteenth Century. However, biological essentiality of zinc was first recognized by Raulin in 1869 for a fungus aspergillus nigar. It's occurence in biological matter was first described by Laucharlier and Belong in 1877 but its presence in various tissues of man and animal was first reported by Lutz in 1936.

The discovery of Keilin and Mann (1942) that zinc forms an integeral part of enzyme carbonic anhydrase explained its mode of action but its essentiality for human beings was established by Prasad et al (1963) following detailed study in Egypt. Since then extensive study has been done and now more than 80 metalloenzymes have been identified which are essential for various metabolic processes (Vallee et al, 1978).

Absorption and Excretion :

Approximately 20-30% of ingested dietary zinc is absorbed. Absorption of zinc, which probably involves active transpart, is thought to occur mainly in the duodenum and proximal small intestine.

Prostaglandins may be involved in the absorption of zinc in the rat since administration of indomethacin was found to lessen the absorption (Halstead et al 1974).

Zinc absorption is variable and it depends on factors like quality of food, body size, level of zinc in the diet and the presence of other potentially interferring substances in the diet as calcium, phytates and chelating agents.

Zinc is excreted in feces, urine and sweat. Drinker et al (1927) reported average daily excretion of 10 mg/day in feces while kidney only excrete between 0.1 to 0.9 mg in 24 hours (Weisman 1976. Irrespective of season zinc loss through sweat is almost constant i.e. 1.15 mg/L, Prasad et al (1963).

Biochemistry of Zinc :

The first reports demonstrating the phenomenology of zinc deprivation in vivo appeared more than a hundred years ago (Raulin J 1869).

One of the most important discoveries concerning the biologic role of zinc occured in 1940. At that time Keilin and Mann found that zinc was an essential component of erythrocyte carbonic anhydrase, an enzyme catalytically involved in the transport of carbon dioxide in blood.

Zinc is essential for the function and/or structure of several dehydrogenases, aldolases peptidases, phosphatases, an isomerase, transphosphorylase, and aspartate trans carbamylase (Vallee et al, 1970).

These enzymes are present throughout all phyla and participate in a wide variety of metabolic processes including carbohydrate lipid, protein and nucleic acid synthesis or degradation.

Zinc also has been present in DNA (Shin et al, 1968) and also in RNA (Prask et al, 1959) has a role in maintaining the structure. A deficiency of zinc in Euglena gracilis has been shown to effect adversly all the phases of cell cycle (C_1, S, G_2 and mitosis) indicating that zinc is required for biochemical processes essential for cells to pass from G_2 to mitosis, from S to G_2 and G_1 to S (Riordan, 1976). The effect of zinc on the cell cycle is due to it's vital role in DNA synthesis (Prasad, 1974; Krischgessner, 1976).

It is also suggested that catabolism of RNA may be regulated by zinc (Terhune, 1972; Scruton, 1971).

Effect of Zinc on cell and membrane :

Zinc prevents, induced histamine release from mast cells (Chwapil, 1976). It is believed that this effect of zinc is due to it's action on the cell membrane.

Distribution of Zinc in Tissues :

Distribution of zinc in human and animal tissues (Tipton and Cook, 1963; Mcbean et al, 1972) highest values have been found in human prostate gland and eye.

Serum and Plasma Zinc :

Serum zinc is 16% higher than plasma (Toley et al, 1968) due to liberation of zinc from platelets during clotting and invisible hemolysis approximately 90% of serum zinc is loosely bound to albumin fraction so reduction in albumin concentration will result in corresponding fall of serum zinc (Weismen, 1976).

Erythrocyte Zinc :

R.B.C.s have 7-8 times higher zinc content than serum. The normal values reported in literature are 10-14 ug/ml red cells (Rose et al, 1958; Prasad et al, 1965) a large percentage of zinc in RBC's forms an integeral part of carbonic anhydrase and other enzymes.

Leucocyte Zinc :

W.B.C.'s contains 25 times as much as of zinc than erythrocytes do. The normal range is 56.8 - 168 ug/ 10^{10} WBC. Eosinophils contain higher zinc than other leucocytes (Wolff et al, 1956).

Interaction of Zinc with other metals :

Zinc is known to compete with cadmium, lead, copper, iron and calcium for similar binding sites (Hill, 1976).

Zinc Deficiency States :

Causes :

(1) Inadiquate dietary intake :

Protein calorie malnutrition in the developing parts of the world is probably the most common cause of zinc deficiency, but estern population may be at risk from a marginal intake. This may be the cause in vegans, vegetarian (Bodzy et al, 1977) low socioeconomic groups subsisting on low meat diets (Hambridge et al, 1976) and patients with chronic renal disease on low protein diets (Rose and Widden, 1972). Diets used in the treatment of children with inborn errors of metabolism and dietary intolerances require zinc supplimentation (Alexander et al, 1974).

(2) Absorption :

Defective absorption of zinc may be secondary to the immaturity of its absorption this may be the cause in newborn babies especially in preterm (Dauncey et al,1977).

In 1973, Moynahan and Barnes made the important discovery that all the clinical manifestations of acro

dermatitis enteropathica (AE) resolved strikingly with oral zinc treatment (Moynahan, 1974). Recently preliminary evidence of defective zinc binding by duodenal juice of patients with AE has been reported (Casey et al, 1978).

(3) Increased body losses in :

e.g. :-

Porphyria	Starvation
Chronic blood loss	Burns
Parasitic infestation	D.M.
Exfoliative dermatitis	Ketoacidosis
Excessive sweating	Diuretic treatment
	Proteinuria
	Hepatic disease
	Intra vascular Hemolysis

(4) Intravenous feeding :

Intravenous feeding if it is prolonged, carries a risk of zinc deficiency.

This is in part secondary to the variable but low content of zinc in the administered solutions (Van Caillie et al, 1978).

Excessive urinary losses of zinc complexed with carbohydrates or amino acids, or both (Freeman et al, 1975). It is particularly likely to occur during an anabolic phase where there is an abrupt increase in body requirements and it is important to monitor zinc status during and after reintroduction of oral feeding (Fleming et al, 1976).

Clinical manifestations of zinc deficiency :

The early features of zinc deficiency are variable and protean. They are anorexia, growth retardation, impaired taste and olfactary sensation and mood alterations. Most neurological features have been described in adults with induced zinc deficiency (Henkin et al, 1975).

Jitteriness and altered behaviaur have been reported in infants (Sivasubramaniam and Henkin, 1978).

The biochemical consiquences of zinc deficiency in human adults include reduced plasma alkaline phosphatase and lactate dehydrogenase activities, raised plasma ammonia, increased plasma ribonuclease activity (Prasad et al, 1978).

Impaired cell mediated immunity with poor lymphoblast response absent skin sensitivity reactions (Golden et al, 1978). Thymic hypoplasia (Golden et al, 1977) and defective monocyte and polymorphonuclear leucocyte mobility have been associated with zinc deficiency and may contribute to the infections which occur particularly in patients with acrodermatitis entropathica (AE) (Weston et al, 1977).

In the pregnant rat even transient zinc deficiency results in an increased incidence of fetal resorption, still births, and abnormalities of CNS, Skeletan, lung, urogenital tract, and palate in off spring. Moreover, the new born rats

tend to be of low birth weights with altered learning and behavioral patterns (Hurley and Mutch, 1973). The one abortion and the two congenital abnormalities reported in the seven recorded pregnancies of three women with acrodermatitis entropathica suggest that similar effects may apply to the pregnant women (Hambridge et al, 1975).

It has been speculated that high incidence of anencephaly in some middle estern countries may be the result of environmental zinc deficiency (Sever and Emanuel, 1973). Serum zinc levels in women having abnormal deliveries or low birth weight or preterm infant were lower than in women not experiencing such abnormalities (Jamson, 1976).

Adolescent nutritional dwarfism :

Zinc deficiency with clinical significantce first came to medical attention in 1963 when Prasad et al described boys from the middle east with a syndrome of poor growth, hypogonadism and anemia. These reports were not the first descriptions of the clinical entity since Lemam had reported a similar group of patients in 1910 whose malady he attributed to parasitic infestation. Turkish workers also described the syndrome, in part over 30 years ago.

Acrodermatitis Enteropathica (AE) :

Characterized by skin lesions, unremitting diarrhoea, and alopecia. It is a rare illness with autosomal recessive inheritance that present during infancy and usually results in death within three years (Walravens P.A. 1979).

Breast feeding of infants delays the onset of manifestations and this is belived to be due to the binding of zinc to a low molicular weight protein that enhances its absorption.

Hypoguesia in children :

Hambridge et al (1972) showed decrease in taste acuity (hypoguesia). Extensive studies by Henkin (1978) have demonstrate that zinc plays a role at several complex stages of the taste process.

Intestinal malabsorption states :

Caggiano and Co-workers (1969) reported a patient with malabsorption and hypogammaglobulinemia whose growth failure responded to zinc therapy.

Acne : Michaelsson et al (1977) carried out a double blind investigations and found that zinc sulfate was as effective as oxytetracycline in the treatment of acne vulgaris.

Hillstrom et al (1977) found that zinc sulfate was better than placebo in the treatment of acne.

Pfeiffer (1978) has claimed a possible connection between zinc deficiency and lack of vitamin B_6 in acne patients.

Sickel cell anaemia :

Prasad et al (1977) found that concentration of the metal was decreased in plasma erythrocytes and hair and that elevated urinary excretion was probably due to either encreased renal filtration of zinc because of continued hemolysis or defect in the tubular reabsorption of zinc.

Nephrotic syndrome :

Reimold et al (1980) found that both plasma and hair levels of zinc were decreased. Plasma zinc increased during remisstion but still remained low.

Prasad (1979) has suggested that the mechanism of zinc deficiency in renal disease includes loss of zinc protein complexes through the glomerulus or by failure of tubular reabsorption.

Zinc and Malnutrition :

Hansen and Lehman (1969) found low levels of plasma zinc in patients with PEM.

Goel and Mishra (1980) found that plasma levels of zinc were significantly lower in all the grades of PEM as compared to the level obtained for control children.

Kumar and Rao (1973) suggests that zinc is mainly bound to albumin, hypoalbuminemia present in patients of PEM also contributes to low zinc levels.

Sharda and Bhandari (1977) zinc is an integral part of DNA and RNA polymerases and its deficiency causes growth retardation in young animals perhaps mediated through an impairment of nucleic acid and protein metabolism.

Alterations in zinc metabolism during infections :

A slight decline in serum albumin is typical of most infectious diseases, especially those that become chronic, but reduction in serum albumin are insufficient to explain the rapid changes in serum zinc studies of alpha-2 macroglobulin, have not shown changes that could explain the depression in serum zinc concentrations during infection (Mcbean et al 1972).

Evidence obtained by radio zinc techniques and direct tissue analysis indicated that the early precipitans depression of serum zinc concentration during infection or inflammatory states can be accounted for in large measure by accelerated flux of zinc from plasma to liver (Powanda et al 1973, Pekarek, 1972).

ZINC AND INFECTIONS :

For more than a century infection induced alterations have been recognized in the metabolism of body content of many substances. With respect to the trace elements, recent reviews have emphasized the consistent occurence during various infections of changes in the serum concentrations and metabolic homeostasis of iron, zinc and copper (Beisel et al 1972 and 1974, Weinberg 1974).

Vikbladh (1951) was the first to report that serum zinc concentrations were reduced below normal in patients with acute infectious illnesses.

Zinc in Tuberculosis :

Halstead and Smith (1970) found that in active tuberculosis both febrile and untreated cases and those on usual drug therapy but still clinically active plasma zinc was significantly low. When the disease became in active the concentration rose. They took 324 cases in the study group and 89 controls. They took 46 cases of active tuberculosis and 8 cases of inactive tuberculosis. The mean values of zinc were 74 $\pm$ 14 and 85 $\pm$ 9 ug/100 ml respectively.

Sharda and Bhandari (1977) had an study on child hood pulmonary tuberculosis they took 27 children suffering from pulmonary tuberculosis. 50 were control. They found that serum zinc levels were markedly decreased in study group than

control group. The mean serum zinc level in pulmonary tuberculosis was 79 ± 12 ug/100 ml and mean serum zinc level in control was 123 ± 23 ug/100 ml.

Bogden et al (1977) : Effect of pulmonary tuberculosis on blood concentrations of copper and zinc they took 30 tubercular patients and 20 controls for estimation of zinc concentration in plasma and 36 patients of tuberculosis and 42 controls for zinc estimation in whole blood. The mean plasma zinc level in tubercular patients was 62 (9.49) ug (u md/L) and in control it was 87 (13.31) ug (u md/L). The mean whole blood zinc concentration in tubercular patients was 556 (85.07) ug (u md/L) and in control 699 (106.95) ug (u md/L).

They interpreted that the whole blood zinc was significantly lower. There was no significant difference in erythrocyte zinc concentrations between the TB patients and controls.

Lindeman and colleagues (1971) have reported no significant effects of age and sex on erythrocyte zinc concentrations. They did find that plasma zinc decreases by about 3 ugm/dl (0.46 umol/l) with each decade of age.

Recent evidence suggests that the sex of the subject has no effect on serum copper levels, and the

effect of age on serum copper level is small with an increase of only 2 ug/dl (0.31 umol/l) with a ten year age increase (Yunice et al, 1974).

Khatri et al (1981) had an study on serum zinc in pulmonary infections. They took 11 patients with tubercular infection and 20 normal healthy controls. The mean serum zinc levels in pulmonary tuberculosis was 74.2 ± 27.21 ug/dl.

Values in patients with tuberculosis were lower than control but the difference was statistically not significant.

Niculescu et al (1981) studied changes of zinc in serum of patients with silico-tuberculosis, and active lung tuberculosis. They took 45 patients with silico tuberculosis, 28 patients with active lung tuberculosis; the T.B. patients were selected as regards their "activity" according to the following criteria : The tubercle bacilli presence in sputum, and radiological, immunological, and clinical criteria. The control group consisted of 40 healthy persons. The mean serum zinc values in silicotuberculosis was 92.27 ± 34.37 ug/100 ml. In active lung tuberculosis mean serum zinc level was 82.06 ± 15.21.

The mean values of zinc in silicotuberculosis and active lung tuberculosis were significantly decreased as compared to controls. The zinc level decreased by 22% with silicotuberculosis group and by 31% in the tuberculosis group Vs the control.

Gupta et al (1984) status of zinc in pulmonary tuberculosis. 30 patients of pulmonary tuberculosis 15 male and 15 female and 20 normal healthy individual 10 male and 10 female of identical age and sex as control.

The severity of tuberculosis was classified by X-ray chest into three stages, according to the criteria by National Tuberculosis Association, USA (1961).

Stage - I : Slight to moderate density without demonstrable cavitation and the area does not exceed the volume of lung, which lies above the second costochondral junction, may be bilateral.

Stage -II : Slight to moderate dence opacity which does not exceed 1/3 the lung volume, may be bilateral and the size of cavity if present must be less than 4 cm.

Stage - III : Lesions which are more extensive than moderately advanced.

The mean serum zinc level in tuberculosis patients was 61.86 ± 9.60 ug% and in control it was 99.8 ± 10.84 ug%. In male tuberculosis patient the mean serum zinc level was 64.13 ± 9.22 and in control 99.3 ± 10.63 ug%. In female tuberculosis zinc level was 59.66 ± 9.44 and in female control 100.3 ± 11.03 ug%.

The serum zinc level in control subjects of different age groups was within normal limits. There was no change in serum zinc level in relation to age and sex. The level of serum zinc was significantly low in both male and female patients having tuberculosis. The 7 cases of stage I were having the mean serum zinc level 69.57 ± 11.81 umg%, 14 cases of stage II were having the zinc level 62.71 ± 6.90 and 9 cases of stage III were having the mean serum zinc level 53.33 ± 3.80 umg%.

Serum zinc level decreases as the severity of disease increases. So in stage III tuberculosis level of zinc markedly reduced.

Sinha et al (1985) reported the importance of serum zinc and copper in pulmonary tuberculosis of childhood. They took 140 cases in the age group of 9 months to 12 years. 40 normal children were taken, to serve as control group.

They categorized tuberculosis into two group active and inactive. Active which was sputum or/gastric aspirate positive for AFB. Inactive which was sputum or gastric aspirate negative.

The mean serum zinc level in active pulmonary tuberculosis was 79.87 ± 12 ug/dl, in inactive pulmonary tuberculosis was 130.35 ± 12.94 ug/dl and in control it was 130.95 ± 22.53 ug/dl.

Serum zinc level was markedly demished as compared to control group. More or less similar values were seen in both control as well as in inactive pulmonary tuberculosis.

There was another study on serum zinc and copper in tuberculosis (Ahmad P. et al, 1985) in which 30 children suffering from tuberculosis and 35 normal healthy age and sex matched children serve as control were taken. In all cases serum zinc was estimated before and 4 weeks after starting chemotherapy.

The mean serum zinc level in tubercular patients before chemotherapy was 63.07 ± 5.4 ug/dl and after chemotherapy was 76.1 ± 3.1 ug/dl, whereas in control 98.66 ± 11.4 ug/dl.

Observations of this study was significant hypozincemia in cases of pulmonary tuberculosis, as compared to normal children. Serial estimation of serum zinc after 4 weeks of antitubercular therapy indicated a significant rise in serum zinc levels (Ahmad P et al, 1985).

Hua (1989) studied serum zinc in 72 patients with pulmonary tuberculosis, 50 healthy person as control. In patients of tuberculosis serum zinc was significantly decreased when compared with controls.

Zinc and non tubercular infection :

Vikbladh (1950) was the first to report that serum zinc concentrations were reduced below normal in patients with acute infectious illnesses. They were taken mainly the patients of pneumonia, bronchitis, pyelonephritis, found the decreased serum zinc levels significantly as compared to control group. His observations had amply confirmed in patients with bacterial, viral, rickettsial, spirochaetal and parasitic infection (Beisel et al 1972, 1974; Halsted et al 1970; Mcbean et al 1972).

Sinha and Gabrieli (1970) had study on serum zinc and copper ~~x~~ levels in various pathological conditions, they took 967 cases of different pathological condition. Out of 967 cases, 38 were of pneumonia rest were of non infectious cases. Control group was comprised of 200 healthy subjects. Author concluded that serum zinc level was markedly decreased in pneumonia, and other pathological conditions of non infectious in origin as compared to control. Mean serum zinc level in pneumonia was 108 $\pm$ 27 ug/100 ml whereas mean serum zinc in control subjects was 120 $\pm$ 22 ug/100 ml.

Srinivas et al (1988) had an study on trace element alterations in infectious diseases. They were included 53 patients which was divided into five different types of infections. 11 patients of septicemia, 14 patients with pneumonia 17 patients of viral infections, 3 patients with acute bacterial meningitis were also studied.

The mean plasma levels of zinc in sepsis was 9.4 ±27 u mol/L, in pneumonia 7.3 ± 2.7, in viral infectious 12.4 ± 1.8, in meningitis 5.2 ± 2.2 u mol/L and in control it was 12.8 ± 1.6 umol/L.

The zinc concentrations were significantly lower in all groups as compared with healthy controls with the exception of patients with viral infection where the values of zinc were not significantly different from healthy controls.

They also analysed trace elements in six patients of sepsis and six patients of pneumonia during recovery from infection (38 ± 19 days for pneumonia and 13 - 8 days for sepsis). Although these values started returning to normal, they still differed significantly when compared with healthy control values.

Khatri et al (1981) studied serum zinc in pulmonary infections. The study was conducted on 5 patients with non tubercular pulmonary infections and 20 normal healthy controls.

Among those with non tubercular pulmonary infections (3 with bronchopneumonia and 2 with lobar pneumonia) 3 were below 2 years and 2 between 2 and 4 years. The mean serum zinc level in non tubercular pulmonary infections was 70.4 ± 15.5 ugm/dl as compared to the normal healthy controls where serum zinc level was 91.2 ± 25.6 ug/dl. The reduction in serum zinc level in nontubercular pulmonary infections was significant.

Zinc and hepatitis :

Serum zinc concentrations during acute infectious hepatitis generally decline to values comparable to those observed in other types of infections (Caveides et al,1964; Halsted et al, 1970; Henkin et al, 1972; Kahn et al, 1965; Karlinskii et al, 1965). Low serum zinc were found in patients of infective hepatitis following hyperzincuria. The zinc in serum was found to exists almost entirely in a diffusible states as compared to 30% to 40% of serum zinc tightly bound to an alpha macroglobulin in normal persons.

Sharda and Bhandari (1977) studied serum zinc concentration in acute viral hepatitis. They were included 20 children 2 to 7 years of age suffering from acute viral hepatitis and 20 normal children as control.

The diagnosis of hepatitis was based on epidemiology, history, clinical examination and elevated serum enzymes. The mean serum zinc level in viral hepatitis was 58 ± 15 ug/100 ml whereas in controls it was 116 ± 20.5 ug/100 ml.

There was no evidence that age, sex affect the serum zinc levels. Serum total proteins, A : G ratio were same as in normal controls; SGPT, alkaline phosphatase and serum bilirubin were elevated in acute viral hepatitis. The mean serum zinc in viral hepatitis was significantly decreased than controls.

Halsted et al (1968) were alos found that plasma zinc was 1-2 SD below the control in patients with viral hepatitis.

A low serum zinc concentration is a non specific finding occuring in many types of liver diseases (Halsted, 1968).

Alone study of Kahn et al (1965) was contrary to the other studies that serum zinc was elevated in 10 patients with viral hepatitis instead of depression of zinc values in hepatitis.

Zinc and Leprosy :

Serum zinc levels were estimated by AAS in 56 leprosy patients, 42 normal healthy subjects as controls. A significant reduction in zinc was noted throughout the leprosy spectrum. Mean serum zinc level in leprosy patients was 74.86 $\pm$ 4.89 ug/100 ml whereas in controls the mean serum level of zinc was 118.0 $\pm$ 27.0 ug/100 ml, Rao et al, (1985).

COPPER :

It has been known for more than 100 years (Underwood, 1977) that copper is a component of hemocyanin, the respiratory pigment of snails. Hart et al (1928) were the first workers to notice that copper has a biochemical function in mammals.

Copper was then found to be essential, along with iron for normal erythropoisis.

Copper is present in serum in atleast two fraction (1) A transport fraction (approximately 5%) losely bound to albumin. (2) Ceruloplasmin (approximately 95%) firmly bound to globulin (Wintrobe et al, 1953; Gulter et al, 1953).

Requirement and Sources :

The daily requirement of copper vary between 0.08 mg/kg of body weight for infants and 0.03 mg/kg for adults (WHO technical series, trace element in human nutrition, 1973).

Meat, fish and green leafy vegetables are rich in copper content. The estimated dietary intake of copper from a typical Indian diet is around 2 mg/day.

Absorption :

Copper by forming complexes with amino acid, is absorbed by active process and some of it is associated with

metallothionine, in the mucosal cell. The copper is transported in the blood to the liver, where ceruloplasmin synthesis takes place (Solomons, 1977).

Excretion :

Copper is excreted through urine, sweat and bile, however, the amount of copper in urine and sweat is very little. The main portion of it is excreted in the bile, major part of which is again absorbed. Turnover studies of Cartweight and Wintrobe (1953) indicate that three fourth or more of faecal copper represents biliary secretion. This amount will be further increased to several folds in the presence of diarrhoea.

BIOCHEMISTRY :

Copper dependent enzymes :

Enzymes are proteins which catalyze specific metabolic reactions and some of them tightly bind a metallic ion which is essential for enzymatic activity, known as cuproenzymes with the exception of tryptophan-2, 3-dioxygenase. Brady et al (1972) reported that this enzyme, purified from both rat liver and pseudomonas acidovorous, contains 2 gram atoms of copper and 2 moles of heme per mole of tetrameric enzyme.

Ishimura and Hayaishi (1973) did not find a stoichiometric quantity of copper in a tryptophan dioxygenase

purified from P.fluorescens. The enzymes that have crucial role in oxidative metabolism. These include :-

(i) Cytochrome oxidase : Terminal oxidase in the electran transport chain the only enzyme that can reduce molecular oxygen to water and one which is necessary for the production of virtually all energy of metabolism (Galaghan, 1973).

(ii) Ferroxidases : including ceruloplasmin, that have an important role in iron metabolism.

(iii) Copper amineoxydases required for the cross linking of elastin and collagen.

(iv) Superoxide dismutase that removes superoxide free radical anions (Mam, 1938; McCard et al, 1969).

(v) Ascorbic acid oxidase.

(vi) Tyrosinase, which is required for the synthesis of melanin.

Metallothionine, another cuproprotein, is currently the subject of considerable discussion and controversy, but appears to play an central role in copper metabolism especially in the liver. Another hepatic curproprotein, mitochondrocuprin, which contains 2 to 4% copper, is specific for the neonatal period and accounts for the large stores of hepatic copper in the term neonate.

Anaemia and Copper :

Iron is absorbed as Fe^{2+} and must be converted to Fe^{3+} to be transported by transferrin. The plasma iron binding protein ceruloplasmin is a ferroxidase is required to catalyse the oxidation of Fe^{2+} at a rate which will allow normal absorption and transport of iron (Osaki, 1966).

Cartwrite et al (1964) and Lee et al (1968) found that the erythrocytes in copper deficient animals have a shorter life span than normal. Copper deficiency is thought to interfere with haem synthesis in three ways :-

(i) By deminishing iron transport into mitochondria (Goodman et al, 1969).

(ii) By inhibiting the activity of ferrochelatase (Trephly et al, 1978).

(iii) By reducing the concentration of the copper iron enzyme cytochrome C oxidase. Hari et al (1928) suggests that copper might be effective in treating anaemic infants.

Copper in Blood :

Wintrobe et al (1953) found that about 49% of the copper in whole blood is in the RBCs where the concentration is about 110 ug/dl of RBCs, unlike the plasma copper there is very little species variation in RBC copper (Evans et al, 1967).

Gubler (1953) reported that in plasma, about 96% of the copper is present as ceruloplasmin and only about 8% of the ceruloplasmin is present as apoceruloplasmin (Matsuda et al, 1974).

Copper in Milk :

The concentration of copper, unlike that of zinc, seems to be no higher in colostrum than in mature mild (Nassi et al, 1974).

Copper deficiency :

Underwood (1971) observed bone defects in rabbits, pigs, chicks and dogs. Rucker et al (1969) observed that copper deficient chick bone contains a higher than normal proportion of soluble collagen and that soluble collagen from deficient bone has less aldihydic function than normal.

Cardiac failure associated with copper deficiency was first observed in Cattle and was termed "falling disease" (Underwood, 1971).

Kelley et al (1974) reported heart failure in young rats whose dams consumed a copper deficient diet.

The integrity of vascular system, particularly of the large arteries, is dependent largely upon the quality and quantity of collagen and elastin in the vessel walls the role of copper in cross linking and maturation process had

been clarified since the simultaneous discovery that pigs and chicks fed copper deficient diets, frequently die suddenly from massive internal heamorrhage caused by structural defects in major arteries (O'dell et al, 1961 and Shields et al,1962).

Copper deficiency during gestation :

Copper deficiency during pregnancy in sheep results in the ataxic disease "swayback" or enzootic "neonatal ataxia" in the newborn lamb, in these affected sheep there was extensive demyelination of the CNS, and the brain mitochondria showed deplition of cytochondrome C oxidase (Underwood et al, 1971).

Widdowson et al (1972) found that at term about half the copper in the body is present in the liver, where it is bound to the protein, recently identified as a metallothionein, (Porter, 1974; and Ryden et al, 1978).

Widdowson (1961) found that after birth, the concentration of copper in the liver falls as the copper is used for growth, though the total amount ultimately increases because of the increase in size of the organ.

Copper and Hereditary diseases :

Menkes'kinky hair disease :

Menkes et al (1962) reported that this is an X-linked recessively inherited disease. This disease apparantly not associated with neutropenia and anemia (Williams et al, 1977) there are pili torti, typical bone changes, arterial intimal changes, seizures with developmental regression, hypothermia and the patients generally die before the age of 2 years. Plasma copper and ceruloplasmin levels are very low (Matsuda et al, 1974; Williams et al, 1977; Danks et al, 1972; Molekaer, 1974; Lott et al, 1975 and Daish et al, 1978). Copper absorption is deffective (Matsuda et al, 1974 and Williams et al, 1977).

Wilson's disease :

Wilson (1972) told that it is an autosomal recessive condition, characterized by progressive cirrhosis of the liver, renal malfunction with aminoaciduria, glycosuria, neurological degeneration and Kayser-fleischer rings in the cornea. These manifestations are due to excessive accumulation of copper in the tissues which result from deminished excretion of copper in the bile (Scheinberg et al, 1976; and Strickland et al, 1972).

Serum ceruloplasmin level is reduced than normal (Gubler et al, 1953).

Evans et al (1973) have isolated a metallothionen with high affinity for copper from cases of Wilson's disease.

Klingbert et al (1976) suggests that zinc deficiency to be avoid during treatment with penicillamine.

Summary of changes in serum copper with age :

Age	Serum copper ug/dl ± SD	Reference
Cord blood	29 ± 11	Henkin et al, 1971
5 days	47 ± 9	
1 month	63 ± 17	
3 month	81 ± 17	Ohtake, 1977
5 month	104 ± 25	
6-12 month	111 ± 19	
6-12 years	109 ± 17	Ohtake & Tamura, 1976

Alteration in copper metabolism during infection :

The declines in serum zinc and iron concentration however, serum copper values tend to increase in conjunction with proportionately higher values of the copper binding protein, ceruloplasmin.

Ceruloplasmin is an alpha-2 globulin with a molecular weight of approximately 1,51,000 and a copper content of 8 atoms per molecule a small fraction of copper in serum is bound loosely to albumin but most is transported by ceruloplasmin (Markowitz et al, 1955).

Changes in copper metabolism during acute infections or inflammatory states are secondary to an increased hepatic synthesis and release of ceruloplasmin (Pekarek et al, 1972; Markowitz et al, 1955). There is accelerated production of many other specific serum proteins during infection or other inflammatory states, proteins of this group have been classified together under the term "Acute phase reactant proteins" and in haptoglobulin, alpha.antitrypsin, fibrinogen, C-reactive protein, seromucoid.

Powanda et al (1972) found that the increases in serum copper and ceruloplasmin concentrations like other acute phase reactant proteins during infection or inflammatory states appear to be mediated by leukocyte endogenous mediator (LEM). The increase in serum copper and ceruloplasmin concentrations persists for a relatively long period of time into convalescence in contrast to more rapid return of zinc and iron values to their individual baseline values. The persistence of high copper and ceruloplasmin concentrations has been attributed to the relatively long half disappearance time of the protein. The increase in both copper

and ceruloplasmin concentrations of serum occurs in acute hepatitis as in other infections (Brendstrup, 1953 and Trip et al, 1969).

COPPER AND INFECTION :

Copper and Tuberculosis :

Bogden et al (1977) : Effect of pulmonary tuberculosis on blood concentrations of copper they took 30 tubercular patients and 20 controls for estimation of copper concentration in plasma and 36 patients of tuberculosis and 42 controls for copper estimation in whole blood. Mean plasma copper in tubercular patients was 162 ± 35 ug/100 ml whereas mean plasma copper in controls was 110 ± 19 ug/100 ml.

The whole blood copper in tubercular patients was 160 ± 36 ug/100 ml whereas in controls it was 112 ± 20 ug/100 ml.

They interpreted that the whole blood copper and plasma copper was significantly higher in the samples from tubercular patients, as compared to controls.

Niculescu et al (1981) studied changes of copper, in serum of patients with silicotuberculosis, and active lung tuberculosis. They took 45 patients with silicotuberculosis, 28 patients with active lung tuberculosis, the T.B. patients were selected as regards their "activity" according to the

following criteria :

The tubercle bacilli presence in sputum and radiological, immunological, and clinical criteria.

The control group consisted of 40 healthy persons.

The increase of the copper values in the silico-tuberculosis and in active lung T.B. is significant 195.11 ± 28.25 mg/100 ml serum representing a 70% increase Vs the controls in the silicotuberculosis group and 209.82 ± 42.69 ug/100 ml (83% increase Vs the controls) in the active tuber-culosis group.

Sinha et al (1985) reported the importance of serum copper in pulmonary tuberculosis of childhood. They took 140 cases in the age group of 9 months to 12 years. 40 normal children were taken as control group.

They categorized tuberculosis into two group - active and inactive.

Active which was sputum or gastric aspirate positive for AFB.

Inactive which was sputum or gastric aspirate Negative.

Mean serum copper in active tuberculosis was 164.1 ± 6.27 ug/dl and in inactive pulmonary tuberculosis it was 108.8 ± 5.62 ug/dl, whereas in controls mean serum copper level was 114 ± 6.67 ug/dl.

Mean serum copper level was increased in active tuberculosis as compared to control group. More or less similar values were seen in both control as well as in inactive pulmonary tuberculosis.

There was another study on serum copper in tuberculosis in which 30 children suffering from tuberculosis and 35 normal healthy age and sex matched children as control. In all cases serum copper and zinc was estimated before and 4 weeks after starting chemotherapy.

Mean serum copper level in tubercular patients before chemotherapy was 150.6 ± 11.5 ug/dl whereas after chemotherapy (4 weeks) it was 137.2 ± 8.7 ug/dl. Mean serum copper level in control was 108.1 ± 8.9 ug/dl.

Observations of this study was significant hypercupermia in cases of pulmonary tuberculosis, as compared to normal children. Serial estimation of serum copper after 4 weeks of antitubercular therapy indicated a significant fall in serum copper levels, Ahmad P. et al (1985).

Hua (1989) studied serum copper in 72 patients with pulmonary tuberculosis and 50 healthy persons as controls. There was significant increase in serum copper level in patients of pulmonary tuberculosis as compared to controls.

Copper and non tubercular infections :

Sinha and Gabrieli (1970) had study on serum copper levels in various pathological conditions, they took 967 cases of different pathological condition. Out of 967 cases, 38 cases were of pneumonia, rest were of non infectious cases. Control group was comprised of 200 healthy subjects. Author was concluded that the serum copper level was increased in pneumonia and other pathological conditions of non infectious in origin as compared to control. Mean serum copper level in pneumonia was 162 ± 36 whereas in controls it was 123 ± 23 ug/100 ml.

Srinivas et al (1988) had an study on trace element alterations in infectious diseases. They took 53 patients which was divided into different types of infections. 11 patients of septicemia, 14 patients with pneumonia.

17 patients of viral infectious. 3 patients with acute bacterial miningitis were also studied.

12 healthy subjects acted as controls.

Mean serum copper level in spesis was 25.1 ± 6.8 u mol/L, in pneumonia 23.0 ± 8.9 u mol/L, in viral 22.8 ± 5.0 u mol/L, in meningitis 21.7 ± 9.2 u mol/L whereas mean serum copper level in controls was 16.6 ± 3.75 u mol/L.

The concentration of copper was high in all groups, it was highest in patients with sepsis as compared with healthy controls.

They also analysed trace elements in six patients of sepsis and six patients of pneumonia during recovery from infection (38 ± 19 days for pneumonia and 13 ± 8 days for sepsis). Although these values started returning to normal, they still differed significantly when compared with healthy control values.

Copper in Leprosy :

Serum copper levels were estimated by AAS in 56 leprosy patients comprising of 14 BT, 12 BB, 11 BL and 19 LL. These findings were evaluated in comparison to 42 normal subjects serving as controls. A signifidant elevation of serum copper was recorded throughout the leprosy spectrum. Mean serum copper level in leprosy patients was 211.73 ± 15.94 ug/100 ml whereas in controls it was 114.0 ± 37.0 ug/100 ml Rao et al (1985).

Pathophysiologic mechanisms that can account for alterations in trace element concentration :

Alterations in the binding of zinc and copper to their respective specific and non specific binding proteins and ligands occurs during infection the accelerated rate of hepatic synthesis of ceruloplasmin appears to account for the increased concentrations of copper observed in serum. In

contrast the early disappearance of zinc from serum appears to occur without appreciable concomitant change in concentration of their respective binding proteins.

In the patients with infectious hepatitis, the zinc in serum seems to exist in an ultrafiltrable form (Henkin and Smith, 1972).

Alterations in body distribution of trace elements during an infection, the accelerated flux of zinc into the liver and the increased output of the copper ceruloplasmin complex from the liver cells, appear to represent physiologic responces initiated by the action of leukocyte endogenous mediator (LEM).

Trace elements are required for the growth and replication of bacteria, fungi and parasites.

Trace element changes are also associated with changes in secondary manifestations of hormonal responces, occur during infection. Infectious process may cause an increased secretion of the adrenal glucocarticoids and mineralocorticoids, ACTH, growth hormone, ADH, thyroid hormones, glucagon and the catecholamines (Beisel, 1967).

It was recently been observed that a substance with hormone like properties is released from phagocytizing while

blood cells into the serum during infectious illnesses (Eddington et al 1971 and 1972; Kampschmidt et al 1973-74; Pekarek et al 1972-74 and Wannemacher et al 1972). This substance has been termed as LEM and has direct action upon the liver causing important effects on the metabolism of zinc and copper (Eddington et al 1971-72; Pakareketal, 1972-74).

Leukocytic Endogenous mediator(LEM):

LEM exerts its principal effects upon the liver but can also stimulate the release of phagocytic cells from the bone marrow (Kampschmidt et al 1973-74).

LEM has been shown to circulate within the serum of man and experimental animals during febrile infections or inflammatory states, (Wannemacher et al 1972).

LEM is also released in vitro by polymorphonuclear leukocytes, macrophages, or monocytes that have been activated by stimulating them to engage in phagocytic activity (Kampschmidt et al 1973-74 and Pekarek et al 1972-74).

ACTION OF LEM ON TRANSPORT SITES :

LEM stimulates the hepatic cells to take up iron, zinc and a large number of free aminoacids from the serum (Kampschmidt et al 1973-74; Pekarek, 1972 and Wannemacher et al 1974).

LEM does not seem to exert its effects via the activation of adenylate cyclase or increased formation of cyclic adenosine monophosphate (CAMP) (Wannemacher et al 1972).

In stimulating an accelerated flux of various substances into the liver, LEM does not appear to function as a metal transport or binding protein by forming bands with either zinc or iron (Pekarek et al 1972).

Neutrophils produce both lactoferrin and LEM. If lactoferrin is released from phagocytizing WBCs it can function as a transport protein in assisting the movement of iron from serum into the liver and other tissues (Masson et al 1969).

LEM stimulation of protein synthesis :

LEM has second series of action on hepatic cells thereby stimulating the accelerated synthesis of nuclear and ribosomal RNA (Wannemacher et al 1972).

It also causes rough endoplasmic reticulum of the cells to accelerate its synthesis of various acute phase reactant proteins (Eddington et al 1971-72; Kampschmidt et al 1973-74; Kekarek et al 1972-74 and Wannemacher et al 1972).

MATERIAL AND METHODS

MATERIAL AND METHODS

The present study was carried out in the Department of Paediatrics, M.L.B. Medical College Hospital, Jhansi U.P. The estimation of serum zinc and copper was done by Atomic Absorption Spectrophotometery in the laboratory of division of Endocrinology at Central Drug Research Institute (CDRI), Lucknow, U.P.

Selection of cases :

A total of 64 cases included in this study were divided into two groups.

Control Group : Ten cases of age and sex matched healthy children without any evidence of severe grade of malnutrition, hepatic, renal disorders or recent infection, were included in this group.

Study Group : Our study group comprised of 54 cases. None of them had previous history of hepatic, renal or other infection depending on the nature of illness this group was further subdivided into bacterial, viral and protozoal infections. The diagnosis was comprised on the basis of history, clinical examination and investigations.

Selection of cases :

The detailed history and clinical examination was done in all cases. Patients with different type of infections were studied. The patients with lobar pneumonia (n = 6) had an X-ray picture consistent with the diagnosis and responded well to antibiotic treatment, raised ESR and leukocytosis.

The patients with Enteric fever (n = 10) had positive widal test. The patients with pyogenic meningitis (n = 6) had raised protein and decreased sugar and increase in polymorphs in CSF, and well responded to antibiotics.

The patients with primary pulmonary tuberculosis (n = 5) had positive Mantaux test raised ESR and lymphocytosis, positive X-ray chest.

The patients with tubercular meningitis (n = 7) had CSF findings consistent with TBM. ESR was raised and differential counts showed lymphocytosis.

The patients of postmeasles bronchopneumonia (n = 5) had positive X-ray chest finding of bronchopneumonia and positive H/O measles.

The patients of infective hepatitis (n = 5) had raised liver function tests e.g. - S.bilirubin, SGOT, SGPT, S. Alkaline phosphatase, Bile salt, Bile pigments.

The patients of Malaria (n = 10) had positive blood film for malarial parasite and well responded to antimalarial treatment.

Each group of infections was consistent with the diagnosis clinically as well as investigation findings.

History : Detailed history of each case was recorded from the parents or attendants on the planned sheet, which included name, age, sex, chief complaints, present illness, relevant past history, family history.

Physical Examination :

General Examination : General condition of the patient, pulse rate, respiratory rate, pallor, cyanosis, clubbing, icterus, lymphadenopathy, oedema, temperature, B.P. any skin changes, hairs.

Anthropometric Examination :

Height, length, weight, head circumference, mid arm circumference.

Systemic Examination :

Thorough systemic examination was done according to disease concerned. Respiratory system, CVS, CNS, abdominal system.

Investigation :

Routine investigations : According to the nature of ailments investigations were selected from the given list.

Blood : Hb%, TLC, DLC, ESR, GBP, MP was done from Department of Pathology of M.L.B. Medical College Hospital, Jhansi.

Urine : Albumin, sugar, microscopic examination, urobilinogen, Bile pigment, Bile salt, culture and sensitivity was done from the Department of Pathology of M.L.B. Medical College, and Hospital, Jhansi.

Mantaux test - done from District T.B. Hospital, Jhansi.

Liver function test : Serum Bilirubin, SGOT, SGPT, Serum Alkaline phosphatase done from Department of Biochemistry, M.L.B. Medical College and Hospital, Jhansi. Estimation of Serum Zinc and Serum Copper of each selected case was done by Atomic Absorption Spectrophotometery (AAS) using PERKIN ELMER 1100B Model from The Division of Endocrinology, CDRI, Lucknow.

Collection of samples :

Five millitres of blood sample was drawn from anticubital vein, using stainless steel needles and acid cleaned, sterilized glass vials which were thoroughly washed with chromic acid and double distilled water. Serum was separated by centrifugation of samples at 3000 r.p.m. for half an hour and places in thoroughly cleaned plain vials. Necessary precautions were taken to avoid contamination. Most of the samples were analysed on the same day, if not then they were kept in deep freeze untill analysed.

Method of estimation of Copper and Zinc :

The estimation of serum zinc and copper was done by the method of Atomic Absorption Spectrophotometry using PERKIN ELMER 1100 B Model. Atomic Absorption Spectrophotometer, installed at the laboratory of Division of Endocrinology, Central Drug Research Institute (CDRI), Lucknow, U.P. Instrumental conditions for measurement of absorbance of the samples are listed in the table given below.

Atomic Absorption Spectrophotometry technique used in the present study was preferred because of its specificity, sensitivity, precision, simplicity and relatively low cost per analysis in comparison to other methods.

Table : Instrumental parameters for measurement of Serum Zinc and Copper by Atomic Absorption Spectrophotometer (PERKIN ELMER 1100 B)

Parameters	Copper	Zinc
Wave length	3247.5°A	2138.6°A
Slit width	100 U	100 U
Lamp current	3 mA	6 mA
Concentration to give 0.25 absorbance	2.3 ug/ml	0.7 ug/ml
Flame	A-A, A-e, A-P	A-A, A-e, A-p
Gas pressure in Tank	10 p.s.i.	10 p.s.i.
Support (air pressure)	15-18 p.s.i.	15-18 p.s.i.
Gas used	Acetylene	Acetylene
Sensitivity	0.04 ug/ml	0.013 ug/ml

Introduction :

In 1860, Kirchoff and Bunsen, observed that the wave lengths of the dark Fraunhofer absorption lines in the solar spectrum coincided with the wave lengths of elemental lines in various emission spectrums. Based upon the assumption that atoms absorb light at the same wave lengths as they emit light, Kirchoff and Bunser deduced the presence of several elements in the solar atmosphere.

In 1955, Walsh showed that this phenomenon of atomic absorption could serve as a spectrochemical basis for quantitative determination of metals. He demonstrated that this measurement of metals by Atomic Absorption Spectrometry are more sensitive than measurements by flame emission spectrophotometery and less subject to interference from other elements. The subject has been comprehensively reviewed by various workers (Allan, 1962; David, 1960; Allan, 1962; Fuwa and Vallee, 1963; Meret and Henkin, 1971; Sunderman,Jr. 1973; Butriomovitz and Purdy, 1977 and Smith Jr et al, 1979).

Principle : Aspiration of the serum into burner produces thermal molecular dissociation and dispersion of metal atoms throughout the flame. Small proportions of these atoms become excited to emit light but the overwhelming majorities of the atoms remain in the ground state and are capable of absorbing discrete wave length of incident light. These specific wave

lengths are provided by a lamp with a hollow cathode. Beam of the light is passed through in flame several times, and then is focussed upon the enterance slit of a differraction grating monochrometer. The absorptions of light at the specific wave lengths are proportional to the concentration of that particular metal in the sample.

The abundance of atoms in the ground state in comparison with those in the excited state accounts for the greater sensitivity of atomic absorption spectrophotometery relative to flame emission spectrophotometery.

Light ermitted from the flame constitutes a potential source of error in AAS, as a light energy which strikes the photomultiplier tube represents the net balance of emission and absorption. This source of error is avoided by modulating the incident light beam with a mechanical chopper, and tunning this photomultiplier detector circuit to the same frequency of modulation, under these conditions, the detector circuit responds only to the pulsed signal from the light beam and does not respond to the continuous signal produced by light emission from the flame. The alternating current from photomultiplier detector circuit is amplified and recorded.

Procedure :

Parker and Colleagues (1967) described two procedures viz simple dilution and removal of protein using trichloroacetic acid prior to aspiration for determination of copper and zinc by AAS. In the present study the simple procedure of direct aspiration was employed. The AAS adjusted to the operating conditions as listed in the table given earlier. Standard solutions for copper zinc were prepared in required strengths copper-1, 2 and 4 part per million; zinc 0.5, 1 and 2 part per million. With these standard solutions of each metal absorption for each solution strength were measured separately and standard curves for each metal were established separately.

After establishment of standard curved serum copper, and zinc were estimated as follows :

Serum samples were aspirated without dilution directly into the flame for copper and zinc. Estimation of absorbance for each metal was done at a time. Duplicate measurements of the absorbance were made for each sample. After every sample measurement distilled water was aspirated into the burner untill null meter returned to null point. Prior to determination of values in the unknown samples,

aliquotes of standard solutions of copper, and zinc were also measured in order to check the precision of the procedure. Serum copper and zinc concentrations were calculated with the help of caliberation curves.

O B S E R V A T I O N S

OBSERVATIONS

A total of sixty four children were included in this study. Out of these 64 cases, ten healthy children acted as control. Their distribution according to age and sex is given in Table I. There was preponderance of male children in the study groups male to female ratio being 1.8 : 1.

TABLE - I : Distribution of children according to age and sex.

Age groups (in years)	Study group		Control group	
	Male	Female	Male	Female
A. 1 to 6 Yrs	30	18	2	4
B. 6 to 12 Yrs	5	1	3	1
Total = 64	35	19	5	5

The study group comprised of children having infectious diseases. Their distribution according to the disease pattern is given in Table II.

Among bacterial infections, 12 cases were tubercular and 22 cases were non-tubercular. Ten cases each of viral and protozoal were included in this group.

Average duration of fever in enteric fever was 10 days and in malaria was 4 days. The cases admitted and included in this study on an average stayed for 7-10 days in hospital. The cases having severe malnutrition, renal pathology, liver disorders (except infective hepatitis), gross oedema, were not included in this study.

TABLE - II : Distribution according to disease pattern.

Type of infections	No. of cases
I. Bacterial Infections :	
(A) Non-tubercular infections :	
1- Enteric fever	10
2- Lobar pneumonia	6
3- Pyogenic meningitis	6
(B) Tubercular infections :	
1- Primary pulmonary tuberculosis	5
2- Tubercular meningitis	7
II. Viral infections :	
1- Post measles bronchopneumonia	5
2- Infective hepatitis	5
III. Protozoal infections :	
1- Malaria	10
Total	**54**

Tubercular bacterial infections :

In all cases of tubercular infections serum values of zinc was significantly lower ($p < 0.01$) as compared to mean value of controls. Mean value of zinc after 7-10 days of therapy was still found to be lower than control ($p < 0.01$), (Table III and V) although it was higher than the mean value observed on zero day. When mean values on 7-10th days compared with mean values of zinc on day zero, found to be rising but rise was insignificant ($p > 0.5$).

While serum copper levels were significantly higher on day zero ($p < 0.01$) and on follow up after 7-10 days of therapy ($p < 0.01$) (Table IV and VI). Serum copper levels on 7-10th day when compared with values on day zero, were found to be decreasing but reduction was insignificant ($p > 0.5$) (Table IV and VI).

TABLE - III A : Serum zinc in primary pulmonary tuberculosis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
Zero day	5	58.9 - 70.9	64.68	5.68
7-10th day	4	60.9 - 76.5	68.27	6.85
Control	10	108.9 -119.7	114.44	4.11

TABLE - III B : Statistical analysis of Table III A.

Groups	d.f.	"t" value	p
A Vs C	13	3.67	< 0.01
B Vs C	12	3.52	< 0.01
B Vs A	6	1.16	> 0.1

d.f. - degree of freedom.

TABLE - IV A : Serum copper in primary pulmonary tuberculosis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	5	111.5 - 120.6	117.78	4.06
B. 7-10th day	4	105.9 - 115.6	110.47	4.01
C. Control	10	95.5 - 100.9	99.27	1.61

ABLE - IV B : Statistical analysis of table IV A.

Groups compared	d.f.	"t" value	p
A Vs C	13	3.60	< 0.01
B Vs C	12	3.29	< 0.01
B Vs A	6	1.77	> 0.1

d.f. = degree of freedom

TABLE - V A : Serum zinc in tubercular meningitis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	7	60.5 - 96.9	76.80	14.02
B. 7-10th day	5	70.9 -100.8	85.92	13.66
C. Control	10	108.9 -119.7	114.44	4.11

TABLE - V B : Statistical analysis of Table V A.

Groups compared	d.f.	"t" value	p
A Vs C	15	3.60	< 0.01
B Vs C	13	3.25	< 0.01
B Vs A	8	0.59	> 0.5

d.f. = degree of freedom.

TABLE - VI A : Serum copper in tubercular meningitis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	7	116.5 - 140.5	126.21	8.39
B. 7-10th day	5	116.5 - 130.9	125.96	8.91
C. Control	10	95.5 - 100.9	99.27	1.61

TABLE - VI B : Statistical analysis of Table VI A.

Groups compared	d.f.	"t" value	p
A Vs C	15	3.67	< 0.01
B Vs C	13	3.50	< 0.01
B Vs A	8	0.68	> 0.5

d.f. = degree of freedom.

BACTERIAL INFECTIONS :

(A) Nontubercular infections :

Mean value of serum zinc (Table VII) on zero day was found to be significantly low ($p < 0.01$) in enteric fever as compared to controls. The mean value of serum zinc on 7-10 day showed a rising trend but was still found to be lower as compared to controls ($p < 0.01$).

TABLE - VII A : Serum zinc in enteric fever.

	Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A.	Zero day	10	57 ~~75~~.5 - 100.8	73.08	17.51
B.	7-10th day	5	68.7 - 108.8	84.44	20.36
C.	Control	10	108.9 - 119.7	114.44	4.11

TABLE - VII B : Statistical analysis of Table VII A.

Groups compared	d.f.	"t" value	p
A Vs C	18	3.76	< 0.01
B Vs C	13	2.95	< 0.01
B Vs A	13	0.75	> 0.1

d.f. = degree of freedom.

Mean serum copper value (Table VIII A) on zero day was found to be significantly higher ($p < 0.01$) in enteric fever as compared to controls. The mean serum values of copper after 7-10 days was still higher as compared to control ($p < 0.01$), although these values were lower as compared to the values on day zero.

TABLE - VIII A : Serum copper in enteric fever.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	10	108.5 - 121.5	118.13	4.83
B. 7-10th day	5	100.5 - 118.9	112.58	7.43
C. Control	10	95.5 - 100.9	99.27	1.61

TABLE - VIII B : Statistical analysis of Table VIII A.

Groups compared	d.f.	"t" value	p
A Vs C	18	4.09	< 0.01
B Vs C	13	3.14	< 0.01
B Vs A	8	1.19	> 0.1

d.f. = degree of freedom.

As depicted in Table IX A, the mean serum value on zero day in lobar pneumonia was found to be significantly lower ($p < 0.01$) as compared to control. The mean serum value of zinc on 7-10th day was still found to be lower as compared to control ($p < 0.01$), although this value was higher as compared to mean value of zinc on zero day ($p > 0.1$).

TABLE - IX A : Serum zinc in lobar pneumonia.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	6	73.5 - 80.9	78.55	2.93
B. 7-10th day	3	82.5 - 90.6	85.86	4.21
C. Control	10	108.9 - 119.7	114.44	4.11

TABLE - IX B : Statistical analysis of Table IX A.

Groups compared	d.f.	"t" value	p
A Vs C	14	3.79	< 0.01
B Vs C	11	3.30	< 0.01
B Vs A	7	1.70	> 0.1

d.f. = degree of freedom.

As is obvious from Table X A, the opposite phenomenon was observed for serum copper levels in lobar pneumonia where significantly higher serum copper level was observed on zero and 7-10 days after therapy.

TABLE - X A : Serum copper in lobar pneumonia.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	6	122.5 - 140.6	132.91	6.85
B. 7-10th day	3	124.9 - 132.5	129.40	3.98
C. Control	10	95.5 - 100.9	99.27	1.61

TABLE - X B : Statistical analysis of Table X A.

Groups compared	d.f.	"t" value	p
A Vs C	14	2.28	< 0.02
B Vs C	11	1.96	< 0.05
B Vs A	7	1.24	> 0.1

d.f. = degree of freedom.

In pyogenic meningitis also (Table XI A), the mean value of serum zinc on zero day was found to be significantly lower ($p < 0.01$) as compared to controls. While mean value of serum zinc after 7-10 day of chemotherapy was found to be still lower as compared to control ($p < 0.01$) but was higher than mean value of serum zinc on zero day ($p > 0.1$).

TABLE - XI A : Serum zinc in pyogenic meningitis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	6	63.6 - 70.9	67.88	2.76
B. 7-10th day	3	70.5 - 71.9	70.96	0.80
C. Control	10	108.9 - 119.7	114.44	4.11

TABLE - XI B : Statistical analysis of Table XI A.

Groups compared	d.f.	"t" value	p
A Vs C	14	3.83	< 0.01
B Vs C	11	3.40	< 0.01
B Vs A	4	2.05	> 0.1

d.f. = degree of freedom.

The opposite results of zinc values was observed for mean serum copper level in pyogenic meningitis too Table XII . Serum copper values remained significantly high from the day zero to subsequent followup.

TABLE - XII A : Serum copper in pyogenic meningitis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	6	122.8 - 140.8	128.73	6.43
B. 7-10th day	3	119.9 - 120.5	120.30	0.34
C. Control	10	95.5 - 100.9	99.27	1.61

TABLE - XII B : Statistical analysis of Table XII A.

Groups compared	d.f.	"t" value	p
A Vs C	14	3.74	< 0.01
B Vs C	11	3.42	< 0.01
B Vs A	4	1.62	> 0.1

d.f. = degree of freedom.

NONTUBERCULAR Vs TUBERCULAR BACTERIAL INFECTIONS :

There was statistically insignificant difference in mean serum values of zinc and copper in nontubercular infections Vs Tubercular infections ($p > 0.5$), Table XIII and XIV).

TABLE - XIII A : Mean serum zinc values in nontubercular (e.g. enteric fever, pneumonia and pyogenic meningitis) and tubercular bacterial infections (primary pulmonary tuberculosis, TBM).

Groups	No.of cases	Nontubercular Mean+SD (kg/100ml)	No.of cases	Tubercular Mean+SD (kg/100ml)	Total Mean ± SD
A. Zero day	22	73.15 ±12.31	12	71.75 ±12.57	72.70 ±12.33
B. 7-10th day	11	81.15 ±14.58	9	77.85 ±13.91	79.67 ±14.01

TABLE - XIII B : Statistical analysis of Table XIIIA.

Groups compared	d.f.	"t" value	p
Zero day	32	0.46	> 0.5
7-10th day	18	0.52	> 0.5

d.f. = degree of freedom.

TABLE - XIV A : Mean serum copper values in nontubercular and tubercular bacterial infections.

Groups	No.of cases	Nontubercular Mean ±SD (µg/100ml)	No.of cases	Tubercular Mean±SD (µg/100ml)	Total Mean ± SD
A. Zero day	22	125.05 ± 8.68	12	122.70 ± 8.54	124.22 ± 8.58
B. 7-10th day	11	119.27 ± 8.87	9	119.07 ± 10.60	119.18 ± 9.42

TABLE - XIV B : Statistical analysis of Table XIV A.

Groups compared	d.f.	"t" value	p
Zero day	32	0.76	7 0.5
7-10th day	18	0.047	7 0.5

d.f. = degree of freedom.

II. VIRAL INFECTIONS :

Mean serum zinc in infective hepatitis (Table XV) was significantly low on day zero ($p < 0.01$) and after 7-10 day ($p < 0.01$) as compared to controls. Mean serum zinc on day zero when compared with mean value of zinc of 7-10 day was found to be insignificant ($p > 0.5$).

TABLE - XV A : Serum zinc in infective hepatitis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	5	55.7 - 64.5	59.72	3.90
B. 7-10th day	3	60.5 - 62.8	61.26	1.32
C. Control	10	108.9 - 119.7	114.44	4.11

TABLE - XV B : Statistical analysis of Table XV A.

Groups compared	d.f.	"t" value	p
A Vs C	13	3.70	< 0.01
B Vs C	11	3.42	< 0.01
B Vs A	6	0.88	> 0.5

d.f. = degree of freedom.

Mean serum copper in infective hepatitis (Table XVI) on day zero was found to be significantly higher ($p < 0.01$) and after 7-10 days was still higher ($p < 0.01$) as compared to controls. Mean copper value on day zero when compared with mean value of copper of 7-10th day was insignificant ($p > 0.5$).

TABLE - XVI A : Serum copper in infective hepatitis.

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	5	124.80 - 126.90	124.80	2.63
B. 7-10th day	3	112.50- 118.80	115.60	3.15
C. Control	10	95.50 - 100.90	99.27	1.61

TABLE - XVI B : Statistical analysis of Table XVI A.

Groups compared	d.f.	"t" value	p
A Vs C	13	3.84	< 0.01
B Vs C	11	3.35	< 0.01
B Vs A	6	2.05	< 0.05

d.f. = degree of freedom.

Mean serum value of zinc in post measles broncho-pneumonia (Table XVII) was found to be significantly lower ($p < 0.01$) on day zero, as compared to controls. After 7-10 days of therapy it was found to be rising towards normal but was still low ($p < 0.1$). The difference between mean value of zinc on zero day when compared with mean value of zinc after 7-10 days of therapy was found to be insignificant ($p < 0.1$).

TABLE - XVII A : Serum zinc in measles (post measles bronchopneumonia).

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	5	76.6 - 84.5	78.68	5.25
B. 7-10th day	4	80.8 - 84.5	81.75	1.83
C. Control	10	108.9 - 119.7	114.44	4.11

TABLE - XVII B : Statistical analysis of Table XVII A.

Groups compared	d.f.	"t" value	p
A Vs C	13	3.63	< 0.01
B Vs C	12	3.51	< 0.01
B Vs A	7	1.10	> 0.1

d.f. = degree of freedom.

Mean serum copper in post measles bronchopneumonia (Table XVIII) was found to be significantly higher than control ($p < 0.01$) on day zero, while after 7-10th day of therapy decreasing trend was found ($p < 0.1$) but was still significantly higher than control. Mean values of copper on day zero when compared with values on 7-10th day found to be insignificant ($p > 0.1$).

TABLE - XVIII A : Serum values of copper in measles (post measles bronchopneumonia)

Groups	No.of cases	Range	Mean (ug/100 ml)	S.D.
A. Zero day	5	122.5 - 130.8	126.26	2.97
B. 7-10th day	4	120.5 - 126.5	123.65	2.58
C. Control	10	95.5 - 100.9	99.27	1.61

TABLE - XVIII B : Statistical analysis of Table XVIII A.

Groups compared	d.f.	"t" value	p
A Vs C	13	3.69	< 0.01
B Vs C	12	3.56	< 0.01
B Vs A	7	1.21	> 0.1

d.f. = degree of freedom.

PROTOZOAL INFECTION :

As in bacterial and viral infections, mean serum value of zinc in malaria (Table XIX) on day zero was found to be significantly lower as compared to control ($p < 0.01$). Mean value after 7-10 days of therapy was still lower than control but was higher than mean value on day zero. Mean value of zinc on 7-10th day was significant when compared with mean value on zero day ($p < 0.02$).

TABLE - XIX A : Serum value of zinc in malaria.

Groups	No.of cases	Range	Mean (ug/100ml)	S.D.
A. Zero day	10	59.7 - 70.5	67.42	3.39
B. 7-10th day	5	70.9 - 74.5	73.06	1.51
C. Control	10	108.9 - 119.7	114.44	4.11

TABLE - XIX B : Statistical analysis of Table XIX A.

Groups compared	d.f.	"t" value	p
A Vs C	18	4.31	< 0.01
B Vs C	13	3.69	< 0.01
B Vs A	13	2.76	< 0.02

d.f. = degree of freedom.

Mean serum copper in malaria (Table XX) was found to be significantly higher on day zero as compared to controls ($p < 0.01$) while after 7-10 days of therapy mean value of copper was found to be decreasing but was still higher than control ($p < 0.01$). Mean value of copper on 7-10th day when compared with mean value of zero day, found to be decreasing but reduction was insignificant ($p > 0.5$).

TABLE - XX A : Serum values of copper in malaria.

Groups	No.of cases	Range	Mean (ug/100ml)	S.D.
A. Zero day	10	110.5 - 125.5	122.50	4.67
B. 7-10th day	5	110.5 - 120.5	117.86	4.15
C. Control	10	95.5 - 100.9	99.27	1.61

TABLE - XX B : Statistical analysis of Table XX A.

Groups compound	d.f.	"t" value	p
A Vs C	18	4.19	< 0.01
B Vs C	13	3.60	< 0.01
B Vs A	13	2.32	> 0.5

d.f. = degree of freedom.

TUBERCULAR INFECTIONS Vs VIRAL INFECTIONS

There was statistically insignificant difference in mean values of serum zinc and copper in tubercular Vs viral infections (Table XXI and XXII), tubercular Vs protozoal infection (Table XXVII and XXVIII), nontubercular Vs viral infection (Table XXIII and XXIV), nontubercular Vs protozoal infection (Table XXV and XXVI) and viral Vs protozoal infections (Table XXIX and XXX).

TABLE - XXI A : Mean serum values of zinc in tubercular and viral infections.

Groups	No.of cases	Tubercular Mean ± SD (mg/100ml)	No.of cases	Viral Mean±SD (mg/100ml)	Total Mean±SD
A. Zero day	12	71.75 ±12.57	10	69.20 ±10.90	70.59 ±11.63
B. 7-10th day	9	77.85 ±13.91	7	72.97 ±11.05	75.71 ±12.58

TABLE - XXI B : Statistical analysis of Table XXI A.

Groups compared	d.f.	"t" value	p
Zero day	20	0.51	7 0.5
7-10th day	14	0.76	7 0.5

d.f. = degree of freedom.

TABLE - XXII A : Mean values of copper in tubercular and viral infections.

Groups	No.of cases	Tubercular Mean +SD (µg/100ml)	No.of cases	Viral Mean+SD (µg/100ml)	Total Mean±SD
A. Zero day	12	122.70 ± 8.59	10	125.53 ± 2.75	123.98 ± 6.60
B. 7-10th day	9	119.07 ± 10.60	7	120.20 ± 5.01	119.56 ± 8.38

TABLE - XXII B : Statistical analysis of Table XXII A.

Groups compared	d.f.	"t" value	p
Zero day	20	1.00	> 0.1
7-10th day	14	0.25	> 0.1

d.f. = degree of freedom.

COMPARISON OF NONTUBERCULAR INFECTIONS WITH VIRAL INFECTIONS

TABLE - XXIII A : Mean values of zinc in nontubercular and viral infections.

Groups	No.of cases	Nontubercular Mean ± SD (γ/100ml)	No.of cases	Viral Mean±SD (γ/100ml)	Total Mean±SD
A. Zero day	22	73.15 ±12.31	10	69.20 ±10.90	71.91 ±11.86
B. 7-10th day	11	81.15 ±14.58	7	72.97 ±11.05	77.97 ±13.60

TABLE - XXIII B : Statistical analysis of Table XXIII A.

Groups compared	d.f.	"t" value	p
Zero day	30	0.87	> 0.5
7-10th day	16	1.24	> 0.1

d.f. = degree of freedom.

TABLE - XXIV A : Mean serum values of copper in non-tubercular and viral infections.

Groups	No.of cases	Nontubercular Mean±SD (µg/100ml)	No.of cases	Viral Mean±SD (µg/100ml)	Total Mean±SD
A. Zero day	22	125.05 ± 8.68	10	125.53 ± 2.75	125.20 ± 7.30
B. 7-10th day	11	121.27 ± 8.87	7	120.20 ± 5.01	119.63 ± 7.44

TABLE - XXIV B : Statistical analysis of Table XXIV A.

Groups compared	d.f.	"t" value	p
Zero day	30	0.17	7 0.5
7-10th day	11	0.29	7 0.5

d.f. = degree of freedom.

NONTUBERCULAR WITH PROTOZOAL INFECTIONS :

TABLE - XXV A : Mean values of zinc

Groups	No.of cases	Nontubercular Mean ± SD (µg/100ml)	No.of cases	Protozoal Mean ± SD (µg/100ml)	Total Mean±SD
A. Zero day	22	73.15 ±12.31	10	67.42 ± 3.39	71.37 ±10.63
B. 7-10th day	11	81.15 ±14.58	5	73.06 ± 1.51	78.62 ±12.54

TABLE - XXV B : Statistical analysis of Table XXV A.

Groups compared	d.f.	"t" value	p
Zero day	30	1.41	> 0.1
7-10th day	14	1.19	> 0.1

d.f. = degree of freedom.

TABLE - XXVI A : Mean values of copper in non-tubercular and protozoal infections.

Groups	No.of cases	Nontubercular Mean±SD (µg/100ml)	No.of cases	Protozoal Mean±SD (µg/100ml)	Total Mean±SD
A. Zero day	22	125.05 ± 8.68	10	122.50 ± 4.67	124.25 ± 7.67
B. 7-10th day	11	121.27 ± 8.87	5	117.86 ± 4.15	118.83 ± 7.58

TABLE - XXVI B : Statistical analysis of Table XXVI A.

Groups compared	d.f.	"t" value	p
Zero day	30	0.87	7 0.5
7-10th day	14	0.83	7 0.5

d.f. = degree of freedom.

TUBERCULAR WITH PROTOZOAL INFECTIONS :

TABLE - XXVII A : Mean value of zinc.

Groups	No.of cases	Tubercular Mean±SD (µg/100ml)	No.of cases	Protozoal Mean±SD (µg/100ml)	Total Mean±SD
A. Zero day	12	71.75 ±12.57	10	67.42 ± 3.39	69.78 ± 9.62
B. 7-10th day	9	77.85 ±13.91	5	73.06 ± 1.51	76.14 ±11.20

TABLE - XXVII B : Statistical analysis of Table XXVII A.

Groups compared	d.f.	"t" value	p
Zero day	30	1.05	> 0.1
7-10th day	12	0.76	> 0.5

d.f. = degree of freedom.

TABLE - XXVIII A : Mean value of copper.

Groups	No.of cases	Tubercular Mean±SD (µg/100ml)	No.of cases	Protozoal Mean±SD (µg/100ml)	Total Mean±SD
A. Zero day	12	122.70 ± 8.59	10	122.50 ± 4.67	122.60 ± 6.89
B. 7-10th day	9	119.07 ± 10.60	5	117.86 ± 4.15	118.64 ± 8.65

TABLE - XXVIII B : Statistical analysis of Table XXVIII A.

Groups compared	d.f.	"t" value	p
Zero day	30	0.06	7 0.5
7-10th day	12	0.25	7 0.5

d.f. = degree of freedom.

VIRAL WITH PROTOZOAL :

TABLE - XXIX A : Mean serum values of zinc.

Groups	No.of cases	Viral Mean±SD (µg%/100ml)	No.of cases	Protozoal Mean±SD (µg%/100ml)	Total Mean±SD
A. Zero day	10	69.20 ±10.90	10	67.42 ± 3.39	68.32 ± 7.91
B. 7-10th day	7	72.97 ±11.05	5	73.06 ± 1.51	73.00 ± 8.21

TABLE - XXIX B : Statistical analysis of Table XXIX A.

Groups compared	d.f.	"t" value	p
Zero day	18	0.5031	7 0.5
7-10th day	10	0.018	7 0.5

d.f. = degree of freedom.

TABLE - XXX A : Mean serum value of copper in viral and protozoal infection.

Groups	No.of cases	Viral Mean±SD (µg/100ml)	No.of cases	Protozoal Mean±SD (µg/100ml)	Total Mean±SD
A. Zero day	10	125.53 ± 2.75	10	122.50 ± 4.67	124.01 ± 4.04
B. 7-10th day	7	120.20 ± 5.01	5	123.65 ± 2.58	119.22 ± 4.63

TABLE - XXX B : Statistical analysis of Table XXX A.

Groups compared	d.f.	"t" value	p
Zero day	18	1.67	7 0.1
7-10th day	10	1.27	7 0.1

d.f. = degree of freedom.

D I S C U S S I O N

DISCUSSION

For more than a century, infection induced alterations have been recognized in the metabolism of body content of many substances. With respect to the trace elements, recent reviews have emphasized the consistent occurence during various infections of changes in the serum concentrations and metabolic homoeostasis of zinc and copper (Beisel et al, 1972; and Weinberg, 1974).

Studies concerning infection induced alterations in zinc and copper metabolism have now advanced beyond the purely descriptive stages.

In the present study we have analysed the serum concentrations of copper and zinc in patients with common acute infections during the initial stages of disease as well as after initiating treatment. The average hospital stay of patient with enteric fever, pneumonia, pyogenic meningitis, hepatitis was about 7-10 days. Therefore, to have uniformity followed up samples for serum copper and zinc levels on 7-10th day.

In the present study, the estimation of serum copper and zinc were done by atomic absorption spectrophotometry (AAS). This method was preferred because of its specificity, sensitivity, precision, simplicity and relatively low cost, per analysis in comparison to other methods.

The mean serum concentration of zinc in control was 114.44 ug/100 ml. This value is comparable to that reported by (Sinha and Gabrieli, 1970; Halstead and Smith, 1970; Srinivas et al, 1988; Sharda and Bhandari, 1977 ; Niculescu, 1981).

The mean serum concentration of copper in control was 99.27 ug/100 ml. This value is comparable to that reported by (Srinivas et al, 1988; Bogden et al, 1977; Ahmad P. et al, 1985).

The mean serum values of zinc and copper in primary pulmonary tuberculosis on the day zero was 64.68 ug/100 ml, which was significantly low ($p < 0.01$), as compared to control value of 114.44 ug/100 ml. While after 7-10 days of therapy, it rose to 68.27 ug/100 ml.

Mean serum value of copper on the day zero was 117.78 ug/100 ml which was significantly higher ($p < 0.01$), than controls (99.27 ug/100 ml). After 7-10 days of therapy the value fell to 110.47 ug/100 ml, which was insignificant when compared with zero day value.

Halstead and Smith (1977), Sharda and Bhandari (1977) observed low level of serum zinc and its altered level as an evidence of active state of pulmonary tuberculosis. Mean serum zinc values of these authors were in accordance with

with the present study. However, Khatri et al (1981), did not demonstrate any appreciable change in serum zinc values. A number of authors (Bogden et al (1977), Ahmad, P. et al (1985), Radhakrishna et al (1985), Niculescu et al (1981) have found a significant hypercupremia and hypozincemia in cases of pulmonary tuberculosis. Their mean serum values for zinc and copper were compatible with that observed in the present study.

In addition to this, Ahmad, P. et al (1985) also estimated serum zinc and copper after 4 weeks of anti-tubercular therapy, they observed a significant fall in serum copper and rise in serum zinc levels but still the levels of both the elements failed to return to normalcy.

As in primary pulmonary tuberculosis, cases of tubercular meningitis had significantly low serum zinc level and increase in copper levels. The reversal trend was evident after starting of therapy.

TABLE - XXXI : Mean values of zinc, copper in various infectious disorders of different authors :

Diseases	ZINC (ug/100 ml)		COPPER (ug/100 ml)		Authors
	Control	Illness	Control	Illness	
Pneumonia	120.00 ± 22.00	108.00 ± 27.00	123.00 ± 23.00	162.00 ± 36.00	Sinha & Gabreili (Serum), 1970.
Pneumonia	96.00 ± 12.00	74.00 ± 13.00	-	-	Halstead & Smith (Plasma), 1970.
Pneumonia	91.20 ± 25.65	70.40 ± 15.55	-	-	Khatri et al (Serum), 1981.
Pneumonia	12.80 ± 1.60	7.30 ± 2.70	16.60 ± 3.73	23.00 ± 8.90	Srinivas et al (Plasma), 1988 (umol/L)
Pyogenic meningitis	12.80 ± 1.60	5.20 ± 2.20	16.60 ± 3.73	21.70 ± 9.20	" "
Tubercular Infections :					
Pulmonary TB :					
- Active	96.00 ± 12.00	74.00 ± 14.00	-	-	Halstead & Smith (Plasma), 1970.
- Inactive	96.00 ± 12.00	85.00 ± 9.00	-	-	" "
- Active TB	130.95 ± 22.53	79.87 ±12.00	114.00 ± 6.67	164.10 ± 6.87	Radhakrishna et al (Serum),1985.
-Inactive TB	130.95 ± 22.53	130.35 ± 12.94	114.00 ± 6.67	108.80 ± 5.62	" "
TB Patients	87.00 ± 12.00	62.00 ± 20.00	110.00 ± 19.00	162.00 ± 35.00	Bogden et al (Plasma), 1977.

Diseases	ZINC (ug/100 ml) Control	ZINC (ug/100 ml) Illness	COPPER (ug/100 ml) Control	COPPER (ug/100 ml) Illness	Authors
Pulmonary TB :					
- Before therapy	98.66 ± 11.40	63.07 ± 5.40	108.10 ± 8.90	150.60 ± 11.50	Ahmad,P. et al (Serum), 1985.
- After 4 wks of therapy	98.66 ± 11.40	76.10 ± 3.10	108.10 ± 8.90	137.20 ± 8.70	" "
Childhood Pul. TB	123.00 ± 23.00	79.00 ± 12.00	-	-	Sharda & Bandar (Serum), 1977.
Active lung TB	118.74 ± 22.49	82.06 ± 15.21	114.25 ± 22.75	209.82 ± 42.69	Niculescu (Serum), 1981.
Pulmonary TB	91.20 ± 25.65	74.20 ± 27.21	-	-	Khatri et al, (Serum), 1981.
Viral infections					
Viral infections	12.80 ± 1.60	12.40 ± 1.80	16.60 ± 3.73	22.80 ± 5.00	Srinivas et al (Plasma), 1988 (umol/L).
Infective hepatitis	116.00 ± 20.50	58.00 ± 15.00	-	-	Sharda & Bhanda (Serum), 1977.

Mean serum value of zinc in lobar pneumonia on day zero was 78.55 ug/100 ml, which was significantly lower than control ($p < 0.01$). After 7-10 days of therapy, it rose to 85.86 ug/100 ml but still remained significantly low. Mean serum level of copper in lobar pneumonia on day zero (132.91 ug/100 ml), was significantly high ($p < 0.01$) than controls. Mean value of copper after 7-10 days of therapy declined (129.40 ug/100 ml), but was still higher than control

Vikbladh (1950), Sinha and Gabreili (1970), Halstead and Smith (1970) made similar observations in cases of pneumonia.

Mean serum zinc in pyogenic maningitis was found to be significantly low (67.88 ug/100 ml, p $<$ 0.01) as compared to controls. While after 7-10 days of therapy it increased to 70.96 ug/100 ml which was still significantly low. Mean value of serum copper on day zero in pyogenic meningitis was 128.73 ug/100 ml which was significantly higher (p $<$ 0.01) than controls, after 7-10 days of therapy mean value of copper became 120.30 ug/100 ml and the fall was insignificant (p $>$ 0.1).

The findings of present study in pyogenic meningiti are in accordance with Srinivas et al (1988).

Mean value of serum zinc in enteric fever on day zero was significantly low 73.06 ug/100 ml as compared to controls. Which after 7-10 days of therapy rose to 84.44 ug/10 ml. Mean serum copper value on day zero was significantly high (118.13 ug/100 ml, p $<$ 0.01) as compared to controls. Mean valu of copper after 7-10 days of therapy was still higher (112.58 100 ml) as compared to controls.

We could'nt find trace element studies in enteric fever.

Mean value of serum zinc in infective hepatitis on day zero was significantly low (59.72 ug/100 ml, p $\angle$ 0.01); after 7-10 days of therapy value rose to 61.26 ug/100 ml, which was still lower than controls. Mean serum copper on day zero in infective hepatitis was 124.80 ug/100 ml as compared to controls, (99.27 ug/100 ml) the difference was significant while after 7-10 days of therapy mean value of copper fall to 115.60 ug/100 ml, which was still higher than controls.

Sharda and Bhandari (1977), Halstead et al (1968) found that plasma zinc was significantly lower than the controls in patients with viral hepatitis. However, a lone study that of Kahn et al (1965) had the contrary findings for serum zinc in hepatitis cases.

Mean value of serum zinc in post measles bronchopneumonia on day zero (78.68 ug/100 ml) was significantly lower than controls and showed an increasing trent (81.75 ug/100 ml) after 7-10 days of therapy. Mean serum copper on day zero was 126.26 ug/100 ml which was significantly higher than control. It decreased to 123.65 ug/100 ml, after 7-10 days.

Mean value of serum zinc in malaria on day zero (67.42 ug/100 ml) and after 7-10 days of antimalarial therapy (73.06 ug/100 ml) was observed to be significantly lower than control (p $\angle$ 0.01). Mean serum value of copper in malaria on

day zero was 122.50 ug/100 ml, was significantly higher ($p < 0.01$) than controls (99.27 ug/100 ml). While after 7-10 days of therapy declined to (117.86 ug/100 ml), which was still higher than control. To the best of our knowledge the infectious studies on acute malaria is not available in literature.

It was interesting to know that inspite of wide variation in symptomatology of various disorders including in the study there was no significant difference in the magnitude of alteration in mean serum zinc and copper levels. Similarly as early as 7-10 days irrespective of nature of infectious disease there was tendency of serum zinc and copper levels to move towards normalcy. In the present study we could not find the comparable observation in the literature.

There was statistically no significant difference in the mean serum value of zinc and copper in various infectious groups, included in the present study. It appears that alterations in serum zinc and serum copper occur in all acute infections and the changes do not seems to be restricted to any specific infection.

The timing and patterns of response for zinc, copper and are influenced by the nutritional state of the host, the duration of infection, the competence of liver

cell functions, and the type of therapy. Despite these variables, the major trace element responces are remarkably consistent and can be ascribed to well defined pathophysiologic control mechanisms. A product of phagocytizing cells, leukocytic endogenous mediator (LEM), acts on the liver to stimulate an accelerated flux of zinc into hepatic cells and to cause an accelerated synthesis of ceruloplasmin. Other mechanisms that may also influence trace element metabolism include altered body balances, sequestration of the elements within tissues, changes in metal binding transport proteins, harmonal actions and trace element uptake by invading organisms.

Many of the essential trace metals like copper, zinc and iron influence the function of the immune system (Chandra and Puri 1985). Deficiencies of these trace elements can have a detrimental influence on the immune response. Deficiency of zinc produces thymic involution, (Golden et al 1977), decreased activity of T helper cells and natural killer cell activity, reduced proliferation of lymphocytes in response to mitogens, and delayed cutaneous hypersensitivity (Chandra and Puri, 1975; Good et al, 1977; Golden et al, 1978).Patients with acrodermatitis enteropathica, are zinc deficient and their neutrophils show an impaired chemotaxis (Mcleon et al 1934). Even marginal copper deficiency can lead to an impaired humoral mediated response (Larsson et al, 1985; Gindler et al, 1972).

Patients with Menkes'kinky hair syndrome have an inborn defect in copper metabolism resulting in low plasma copper levels and are more susceptible to infections (Larsson et al, 1985; Gindler et al, 1972).

A number of infectious diseases caused by bacteria, viruses, rickettsiae, spirocheats and parasites have been known to evoke hypozincemia (Greenblatt, 1979; Ljunghall et al 1977; Payne et al 1973; Pain et al, 1975).

Trauma can also induce alterations in the metabolism of zinc and copper (Greenblatt, 1979).

Interleukin-1 released by activated phagocytes has been postulated to be responsible for these changes (Palmer et al 1987; Sorva et al, 1987). Administration of interleukin-1 produces an increase in liver zinc (Sorva et al 1987) and reduced levels of copper (Greenblatt 1979; Palmer 1987). Although previous studies have dealt with the influence of a variety of infectious diseases on trace element concentration these exists a need for more detailed documentation in clinical condition as to the extent and duration of changes.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study "THE STUDY OF SERUM ZINC AND COPPER ALTERATIONS IN INFECTIOUS DISEASES", was carried out over a period of one year in the Department of Paediatrics, M.L.B. Medical College and Hospital, Jhansi, U.P. and Central Drug Research Institute (CDRI), Lucknow with the following main objectives.

1- To study the levels of serum copper and zinc in infectious diseases in Bundelkhand region.

2- To find out the correlation of levels of serum copper and zinc during the recovery phase (7-10 days after starting chemotherapy). Estimation of copper and zinc were carried out by atomic absorption spectrophotometery technique (Model - PERKIN ELMER1100 B) in the Division of Endocrinology, CDRI, Lucknow, U.P.

Besides estimation of these elements, a thorough general, physical and systemic examination of each case was conducted and investigations were carried out according to disease concerned.

We excluded the patients of infectious diseases who were having severe grade of malnutrition, renal disorders, liver disorders (except cases of infective hepatitis), gross oedema.

Cases were categorised into two groups :

(i) Study group

(ii) Control group

Study group comprised of 54 children and 10 age and sex matched healthy controls. Cases selected were between the age group of 1 year to 12 years. Study group categorised into three according to pattern of diseases.

(i) Bacterial

(ii) Viral

(iii) Protozoal

(i) Bacterial group further divided into two :

(1) <u>Tubercular</u> :

e.g. (i) Primary pulmonary tuberculosis - 5

(ii) Tubercular meningitis - 7

(2) <u>Non-tubercular</u> :

e.g. (i) Enteric fever - 10

(ii) Lobar pneumonia - 6

(iii) Pyogenic meningitis - 6

(ii) Viral infections divided into two groups :

e.g. (1) Infective hepatitis - 5

(2) Postmeasles bronchopneumonia 5

(iii) Protozoal infections : e.g. Malaria- 10

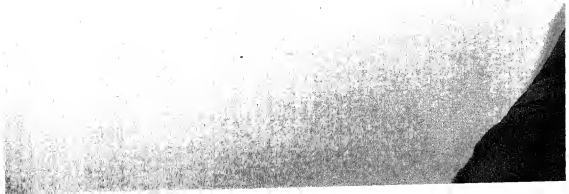
Serum Zinc and Copper :

(1) Mean serum levels for zinc and copper in controls were 114.44 ± 4.11 ug/100 ml and 99.27 ± 1.61 ug/100 ml respectively. These values were compatible with mean values reported in the recent literature.

(2) Mean serum zinc value in primary pulmonary tuberculosis was significantly low (64.68 ± 5.68 ug/100 ml) as compared to controls which after 7-10 days of antitubercular therapy rose to 68.27 ± 6.85 ug/100 ml but still remained significantly lower than control. Statistically there was insignificant difference between the mean value of zinc on day zero and after 7-10 days of antitubercular therapy ($p > 0.1$).

Mean serum copper value on the day zero was significantly higher (117.78 ± 4.06 ug/100 ml) as compared with controls which fell to 110.47 ± 4.01 ug/100 ml after 7-10 days but was still higher than controls. Fall was insignificant.

(3) Mean serum zinc value in tubercular meningitis was significantly lower (76.80 ± 14.02 ug/100 ml) as compared to controls. After 7-10 days of chemotherapy it rose to 85.92 ± 13.66 ug/100 ml. This rise was statistically insignificant ($p > 0.5$).



Mean serum copper value on the day zero was significantly higher (126.21 ± 9.39 ug/100 ml) than controls; after 7-10 days of chemotherapy decreasing trend was found (125.96 ± 8.91 ug/100 ml) but this reduction was insignificant ($p > 0.5$).

(4) Mean serum zinc in enteric fever on the day zero was significantly lower (73.08 ± 17.51 ug/100 ml) as compared to controls. But after 7-10 days of treatment rose to 84.44 ± 20.36 ug/100 ml. The rise was insignificant ($p > 0.1$).

Mean serum copper value on the day zero was significantly higher (118.13 ± 4.83 ug/100 ml) as compared to controls; after 7-10 days of treatment value was found to be decreasing but this decrease was insignificant ($p > 0.1$).

(5) Mean serum zinc in lobar pneumonia was found significantly lower (78.55 ± 2.93 ug/100 ml) than control value, after 7-10 days of chemotherapy it rose to 85.86 ± 4.21 ug/100 ml. This rise was insignificant. Mean serum copper value on the day zero was 132.91 ± 6.85 ug/100 ml which was significantly higher than controls. The value was found to be decreasing after 7-10 days of treatment, but the reduction was insignificant ($p > 0.1$). Values were similar as in the literature.

(6) Mean serum zinc in pyogenic meningitis on the day zero was significantly lower (67.88 ± 2.76 ug/100 ml) as compared to controls. The value rose to 70.96 ± 0.80 ug/100 ml after 7-10 days but was still lower than controls. The rise was insignificant (p $\nearrow$ 0.1).

Mean serum copper on the day zero was 128.73 ± 6.43 ug/100 ml, and the decreasing trend was observed (120.30 ± 0.34 ug/100 ml) after 7-10 days but the reduction was insignificant (p $\nearrow$ 0.1).

(7) In infective hepatitis the mean serum value of zinc on the day zero was significantly lower (59.72 ± 3.90 ug/100 ml) as compared with control. This was found to be rising after 7-10 days (61.26 ± 1.32 ug/100 ml), but the difference was statistically insignificant (p $\nearrow$ 0.5).

Mean serum copper value on the day zero was 124.80 ± 2.63 ug/100 ml which was significantly higher than control; after 7-10 days the value fell to 115.60 ± 3.15 ug/100 ml, the difference being significant (p $\swarrow$ 0.05).

(8) Mean serum zinc in postmeasles bronchopneumonia was significantly lower (78.68 ± 5.25 ug/100 ml) on day zero when compared with controls. It rose to 81.75 ± 1.83 ug/100 ml after 7-10 days of treatment, but the difference was insignificant (p $\nearrow$ 0.1).

Mean serum copper on day zero was 126.26 ± 2.97 ug/100 ml which was significantly higher than control. The decreasing trend was found after 7-10 days (123.65 ± 2.58 ug/100 ml), the difference was not significant (p > 0.1).

(9) In malaria, the mean serum zinc value was also significantly lower (67.42 ± 3.39 ug/100 ml) as compared to controls. It rose to 73.06 ± 1.51 ug/100 ml after 7-10 days of antimalarial therapy, rise was statistically significant (p < 0.02).

Mean serum copper on day zero was 122.50 ± 4.67 ug/100 ml which was significantly higher than control after 7-10 days of treatment fall to 117.86 ± 4.15 ug/100 ml, but the difference was no significant (p > 0.5).

(10) Statistically there was insignificant difference (p > 0.5) between tubercular Vs non tubercular, tubercular Vs viral infections, tubercular Vs protozoal infections, nontubercular Vs viral infections, nontubercular Vs protozoal infectious and viral Vs protozoal infections.

Therefore it could be concluded that :

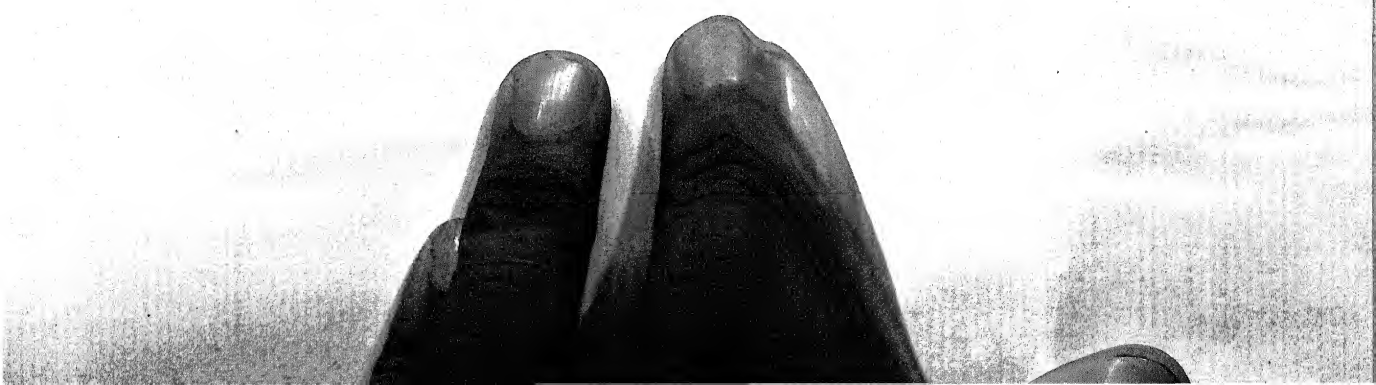
(1) We have excluded the cases who were having severe grade of malnutrition, massive oedema, liver disorders(except hepatitis), renal disorders. Therefore the alterations in the serum zinc and copper values in the present study were due to infections.

(2) In general our findings were at par with those of recent studies in literature.

(3) A significant alterations in the serum zinc and copper were observed in both acute as well as in chronic infections. In general there was fall in serum zinc values and rise in serum copper values.

(4) There was tendency for serum zinc and copper to move towards normal values in both acute and chronic infections. After 7-10 days of initiation of therapy though the value still remains significantly altered.

(5) The magnitude of alteration was same in both acute and chronic infections prior to initiation of therapy. Hence various types of infections can not be differentiated by simply estimating the serum zinc and copper.



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A P P E N D I X

APPENDIX

P R O F O R M A

STUDY ON SERUM ZINC AND COPPER ALTERATIONS IN INFECTIOUS DISEASES

Place of work : Department of Paediatrics,
M.L.B. Medical College and Hospital, Jhansi, U.P.

S.No. _____

Patient's Name _____ **Age/Sex** _____

Father's Name & Address _____

_____

Occupation _____

S.E. Status _____

Family : No. of adults _____

No. of below 12 years _____

Water supply : Tap _____ **Well** _____

Housing : Kachcha _____ **Pakka** _____

Lavatory _____

History _____

Chief complaints : _____

_____

_____

History of Present illness :

Relevant Past History :

Family History :

History of Immunisation :

- B.C.G. _____
- D.P.T. _____
- Polio _____
- Measles/MMR _____

EXAMINATION :

General Examination :

G.C.	_____	Icterus	_____
P.R.	_____	Cyanosis	_____
R.R.	_____	Pallor	_____
B.P.	_____	Lymphadenopathy	_____
Temperature	_____	Clubbing	_____
Hydration	_____	A/F	_____
Oedema	_____	Skin	_____

SYSTEMIC EXAMINATION :

1. **Respiratory System :**

2. **Cardiovascular System :**

3. **Nervous System :**
   - Cerebral Functions _____
     _____
   - Cranial Nerves _____
     _____
     _____
   - **Motor System :**
     - Bulk _____
     - Tone _____
     - Power _____

Reflexes :

- DTR _____
- Superficial Reflexes : _____
  - Abdominal _____
  - Cremastric _____
  - Glutial _____
  - Corneal _____
  - Planter _____
  - Pupils _____

Sensory System : _____

Sign's of Meningeal irritation :

- N.R. _____
- Kernig's sign _____

4. **Abdominal Examination :**
   - Liver _____
   - Spleen _____
   - Ascitis _____

5. **Musculo-skeletal System :**

6. **Genito-urinary System :**

Clinical Impression _____

INVESTIGATIONS :

(A) Routine Investigations :

Blood : TLC _____ DLC : - P _____
ESR _____ - L _____
Hb% _____ - E _____
GBP _____ - M _____
_____ - B _____

Urine : - Routine _____

- M/E _____

- C/S _____

Widal's Test : _____

Sputum for AFB _____

Mx Test _____

X-ray Chest - PA View _____

_____

X-ray Skull - AP _____

- Lateral _____

Liver function tests :

S.Bilirubin _____ SGOT _____

SGPT _____ Alk.Phosphatase _____

Bile Salt _____ Bile Pigments _____

C.S.F. - **Cytobiochemical Examination** :

- Pressure _____
- Colour _____
- Proteins _____
- Sugar _____
- Chloride _____
- Cells _____
- Culture and Sensitivity _____

(B) Serum Zinc and Serum Copper :

Serum Zinc on day zero _____ After 7-10 days _____

Serum Copper on day zero _____ After 7-10 days _____